BRAIN
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Expanded clinical phenotype spectrum correlates with variant function in *SCN2A*-related disorders

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SCN2A-related disorders secondary to altered function in the voltage-gated sodium channel $Na_v1.2$ are rare, with clinically heterogeneous expressions that include epilepsy, autism and multiple severe to profound impairments and other conditions. To advance understanding of the clinical phenotypes and their relationship to channel function, 81 patients (36 female, 44%, median age 5.4 years) with 69 unique *SCN2A* variants were systematically phenotyped and their $Na_v1.2$ channel function systematically assessed. Participants were recruited through the FamileSCN2A Foundation.

Primary phenotype (epilepsy of neonatal onset, $n = 27$; infant onset, $n = 18$; and later onset $n = 24$; and autism without seizures, $n = 12$) was strongly correlated with a non-seizure severity index ($P = 0.002$), which was based on presence of severe impairments in gross motor, fine motor, communication abilities, gastrostomy tube dependence and diagnosis of cortical visual impairment and scoliosis. Non-seizure severity was greatest in the neonatal-onset group and least in the autism group ($P = 0.002$). Children with the lowest severity indices were still severely impaired, as reflected by an average Vineland Adaptive Behavior composite score of 49.5 (>3 standard deviations below the norm-referenced mean of the test). Epileptic spasms were significantly more common in infant-onset (67%) than in neonatal (22%) or later-onset (29%) epilepsy ($P = 0.007$). Primary phenotype was also strongly correlated with variant function ($P < 0.0001$); gain-of-function and mixed function variants predominated in neonatal-onset epilepsy, shifting to moderate loss of function in infant-onset epilepsy and to severe and complete loss of function in later-onset epilepsy and autism groups. Exploratory cluster analysis identified five groups, representing: (i) primarily later-onset epilepsy with moderate loss-of-function variants and low severity indices; (ii) mostly infant-onset epilepsy with moderate loss-of-function variants but higher severity indices; and (iii) late-onset and autism only, with the lowest severity indices (mostly zero) and severe/complete loss-of-function variants. Two exclusively neonatal clusters were distinguished from each other largely on non-seizure severity scores and secondarily on variant function.

The relationship between primary phenotype and variant function emphasizes the role of developmental factors in the differential clinical expression of *SCN2A* variants based on their effects on $Na_v1.2$ channel function. The non-seizure severity of *SCN2A* disorders depends on a combination of the age at seizure onset (primary phenotype) and variant function. As precision therapies for *SCN2A*-related disorders advance towards clinical trials, knowledge of the relationship between variant function and clinical disease expression will be valuable for identifying appropriate patients for these trials and in selecting efficient clinical outcomes.

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Introduction

SCN2A was first associated with self-limited familial (inherited) neonatal–infantile epilepsy (SeLFNIE), previously called 'benign familial neonatal–infantile seizures' or BFNIS.[1-3] About 10 years later, *SCN2A* was associated with a rare and severe epilepsy syndrome, epilepsy in infancy with migrating focal seizures (EIMFS).[4] Subsequently, a broader spectrum of phenotypes associated with *de novo SCN2A* variants was recognized.[5] The more severe disorders often feature neonatal-onset epilepsy, epilepsy with migrating focal seizures, infantile spasms, later-onset epilepsy, autism spectrum disorder or intellectual disability (ASD/ID) without epilepsy.[4-6] Numerous other neurological and medical morbidities, including hypotonia, dystonia, ataxia, poor eye contact, dysautonomia, scoliosis, cortical visual impairment (CVI) and need for a gastrostomy tube are also reported.[5-14] The severity of impairments and the frequency with which they co-occur in affected individuals are not, however, consistently documented. With the exception of SeLFNIE, virtually all affected individuals exhibit moderate to severe ID. Based on scores from the Vineland Adaptive Behavior Scales, Second Edition (Vineland-2) collected by the Simons Foundation Autism Research Initiative, individuals diagnosed with *SCN2A*-related disorders (*SCN2A*-RD) were found to have adaptive function scores approximately 3 standard deviations (SD) below the norm-referenced mean or in <0.5th percentile for the general population, indicating severe to profound impairment.[13]

The functional consequences of *SCN2A* variants exhibit a broad correlation with clinical phenotype.[8,14-18] Based on a limited subset of known *SCN2A* alleles, variants that induce a gain-of-function (GOF) of the $Na_v1.2$ channel are associated with SeLFNIE and early-onset (<3 months) epileptic encephalopathy, whereas variants that cause a loss-of-function (LOF) are associated with later-onset epilepsy or ASD/ID without epilepsy.[5,8,17] However, the relationship between variant dysfunction and clinical phenotype is probably more complex, and the correlation between channel function and severity of non-seizure phenotypes has not been investigated thoroughly. In the Simons Foundation Autism Research Initiative cohort, relative to those with ASD/ID, individuals with epilepsy had more medical morbidities (e.g. CVI, gastrostomy tube dependence) and significantly lower Vineland-2 scores. Scores in the ASD/ID group were still extremely low, indicating substantial impairment. The functional consequences of *SCN2A* variants in this cohort were not determined.[13]

To advance our understanding of the relationship between *SCN2A*-RD phenotype and the impact of variants on $Na_v1.2$ channel function, we exploited a strategic partnership between the Channelopathy-associated Epilepsy Research Center without Walls (CWoW) funded by the National Institute of Neurological Diseases and Stroke (NINDS) and the *SCN2A* Clinical Trials Readiness Study (CTRS), funded by the FamilieSCN2A Foundation. The CWoW was dedicated to determining the functional impact of epilepsy-associated pathogenic variants on ion channel function. The CTRS was designed to provide systematically collected information about clinical phenotypes and evaluate potential clinical outcome assessment measures that might be adopted or adapted in trials of new precision therapies. In this study, we sought to improve understanding of the frequency and severity of both seizure and non-seizure phenotypes associated with *SCN2A*-RD and to provide a more comprehensive understanding of the relationship between variant function and clinical phenotype.

Materials and methods

Data sources

The clinical data are from the *SCN2A* CTRS sponsored by the FamileSCN2A Foundation. Parents from the international SCN2A community were recruited through Foundation outreach (from April to November 2021). Eligibility required sufficient proficiency in English to participate in the study and evidence of a pathogenic or likely pathogenic *SCN2A* variant. Families residing in North, South and Central America, Europe, Israel and Australia were eligible to participate.

All information about the *SCN2A*-RD-affected individuals ('participants') was reported by parents, with the exception of the variants for which a copy of the genetic testing report was required. The CLIRINX platform[19,20] was used to create a secure, user-friendly, web-based survey portal through which parents could register for the study, upload genetic test reports and complete on-line data-collection forms. Most families participated in the

Vineland Adaptive Behavior Scales, Third Edition (Vineland-3) comprehensive interview, which was administered through web-based conferencing software by trained clinical psychology graduate students (L.E., A.J.E.K., K.P. and A.N.N.), who were supervised by a licensed clinical psychologist (E.A.).

Primary phenotype

We distinguished *SCN2A*-RD with and without epilepsy. For those with epilepsy, we further distinguished participants by age at seizure onset. A seminal study in 2017 introduced the distinction between earlier (<3 months) and later (≥3 months) onset of seizures. This distinction has carried forward in the literature. *A priori*, we took a traditional, child neurology (developmental) approach and specifically distinguished neonatal-onset epilepsy (<1 month[21]), infant onset (1–11 month) and later onset (≥12 months).

Non-seizure phenotype

The data-points collected from parents were determined based upon prior extensive qualitative work and surveys with parents of children with various types of neurodevelopmental disorders, including *SCN2A*-RD.[22-24] Furthermore, the research team worked closely with the leadership and parent-volunteers from the FamileSCN2A Foundation to assure that critical outcome domains of interest were addressed and that questions and instruments used for data collection were appropriately worded and relevant. The CTRS built on the experience of the team in using several assessment measures to characterize developmental and functional impairment in >300 children with different kinds of developmental and epileptic encephalopathies.[22-24] These assessments were adopted directly or adapted from the rehabilitation literature.[25] Because some of these measures are applicable only for ages 2 years and above, we used alternative metrics for 12- to 23-month-olds. Children <1 year of age were not included for ability-based phenotyping purposes because adequate parent-reported measures were not available.

Gross motor

Based on the Functional Motor Scale for those ≥2 years old,[26,27] participants were classified as having severe motor impairment if they depended on a wheeled mobility device for distances of <5 m. For children 12–23 months old, we relied on the parent report of 'severe' gross motor delay.

Communication

Participants were considered to have severe communication impairments if rated as 'seldom or never communicates effectively with familiar people' (the two most impaired levels) on the Communication Function Classification System.[28] For children 12–23 months old, we relied on the parent report of 'severe' communication delay.

Hand use

Investigators developed questions with parents to determine whether their child had any purposeful hand grasp to manipulate objects. Children with no purposeful hand grasp were considered to have severe fine motor impairment.

Eating

Children who were entirely gastrostomy tube dependent were classified as severely impaired in this domain for phenotyping purposes.

Other morbidities

In addition, a number of medical morbidities frequently reported in association with *SCN2A*-RD and highlighted as concerns by parents were queried, particularly whether the child had a diagnosis of scoliosis or CVI.

Severity index

An initial severity index was created by summing the number of domains with severe impairment and the presence of CVI and scoliosis (range zero to six). This was used as a reflection of the severity of 'encephalopathy'. As explained in the 'Results' section, a separate severity index excluding communication was also created for use in the cluster analysis.

To provide a greater appreciation for the levels of impairment in this group, even for those not meeting criteria for severe impairment in each area, we determined the mean standardized Vineland-3 comprehensive interview scores by level of the six-point severity index.[29] Families that participated in a reduced version of the CTRS did not complete Vineland interviews.

Participants <1 year old ($n = 4$) or excluded for missing data ($n = 2$) are described with respect to their Centers for Disease Control (CDC) milestones and parents' assessments of development (Supplementary Table 1).

SCN2A variant function

The functional properties of *SCN2A* variants reported in this study were determined by automated patch-clamp recording of recombinant human $Na_v1.2$ heterologously expressed in HEK293T cells (CRL-3216; American Type Culture Collection).[30] About half of the non-truncating variants were studied in both the canonical, adult brain-expressed splice isoform ($Na_v1.2A$; NCBI accession number NM_021007) and the neonatal brain-expressed splice isoform ($Na_v1.2N$; NCBI accession number NM_001371246). For this study, we used HEK293T cells without co-expressed exogenous β subunits. The methods were fully described previously,[30] and a summary is provided in the Supplementary material.

Scoring and classification of variant function

We classified each variant into one of five categories [GOF, severe LOF, moderate LOF (or LOF), mixed function and wild-type-like (WT-like)] based upon the measured functional parameters.[30] All classifications were made in a blinded manner to all information about the clinical phenotype. For variants with current density <25% of wild-type (WT), other biophysical parameters could not be assessed accurately or reliably, and these variants were classified as severe LOF.

Variants with only one significantly abnormal property were classified based on that single property. For variants with more complex patterns of dysfunction, we used the sum of points assigned to each of the measured parameters to determine a classification score (Table 1). Parameters reflecting current amplitude and voltage-dependent properties received 1 point for GOF, −1 for LOF, or 0 for WT-like. The five other parameters (window current, recovery and onset of inactivation, frequency-dependent channel run-down, and ramp currents) received a point value of 0.5 for GOF, −0.5 for LOF, and 0 for WT-like. Variants with an aggregate score >0 were designated as GOF, whereas variants with a score <0 were LOF. We assigned 'mixed' function when the sum of individual parameter values cancelled each other, giving a score of 0. For

Table 1 Rubric for classifying *SCN2A* variant function

Parameter	Gain of function	Loss of function	Point value
Current density (% WT)	Larger than WT	Smaller than WT	±1
Persistent current (% peak)	Larger than WT	–	+1
ΔActivation $V_{½}$	Hyperpolarized	Depolarized	±1
ΔInactivation $V_{½}$	Depolarized	Hyperpolarized	±1
Window current (% WT)	Larger than WT	Smaller than WT	±0.5
Recovery from inactivation (mutant:WT ratio)	Smaller than 1.0	Larger than 1.0	±0.5
Frequency-dependent rundown (% WT)	–	<95% residual current	−0.5
Inactivation time constant (mutant:WT ratio)	Larger than 1.0	Smaller than 1.0	±0.5
Ramp current (% WT)	Larger than WT	Smaller than WT	±0.5

$V_{½}$ = voltage at half-maximum; WT = wild-type.

variants studied in both adult and neonatal splice isoforms, we adjudicated variant function as mixed when there was a divergent result between the two. Severe LOF variants were assigned a score of −4, which was more negative than any other individual variant score in this study.

Analyses

Descriptive analyses focused on medians with interquartile ranges (IQR) and proportions. Bivariate analyses were performed with non-parametric techniques, including χ^2 tests, Spearman ρ correlations, and Kruskal–Wallis tests for comparison of medians across multiple groups. We used hierarchical cluster analysis with the average method and trim factor of 5% to explore the multivariate relations of the primary phenotype, the impact on variant function and the phenotype severity index. For these analyses, all three variables were treated as ordinal and rescaled from 0 to 10. Mixed and WT-like variants were given the same value because neither was clearly GOF or LOF. Truncating variants and whole-gene deletions were considered as more severe than severe LOF missense variants and characterized as 'complete LOF'. Metrics including the cubic clustering criterion semi-partial R^2 were used to identify an optimal clustering solution.

Ethics review

Initial review by the Lurie Children's Hospital Institutional Review Board (2021-4250, 1/26/2021) resulted in an exempt determination. Subsequent review by the North Star ethics review board also resulted in an exempt determination (#NB200048, 1/13/2022). Consent was obtained in accordance with the Declaration of Helsinki.

Results

Eighty-one individuals [36 (44%) female, median age 5.4 years, interquartile range (IQR) 3.4–9.6, maximum 29 years] with pathogenic or likely pathogenic *SCN2A* variants were included. Families were from North America (58), UK (5), Israel (3), Europe (11), South America (3) and Australia (1).

Sixty-nine (85%) participants had a history of epilepsy. The median age at seizure onset was 5.5 months (IQR 1 day to 16 months, maximum = 108 months). The primary phenotypes defined by presence of epilepsy and age at seizure onset were neonatal onset (<1 month, $n = 27$), infant onset (1–11 months, $n = 18$), later onset (≥12 months, $n = 24$), and ASD/ID without epilepsy ($n = 12$). The age range in the ASD/ID group was 2.2–14.6 years, median 4.7. The youngest age in this group, 2.2 years, was greater than the age at initial seizure presentation for 88% of those who had epilepsy.

In the neonatal-onset group, seizures first occurred on Days 1, 2 and 3 of life, with the exception of one child who was born 6 weeks premature and had the first seizure on Day 19 of life.

Primary and non-seizure phenotypes

For 65 participants assessed with the Vineland-3, the median adaptive composite standardized score was 34 (IQR 26–46, maximum = 74). By primary phenotype, the scores were similar ($P = 0.56$), with median scores of 34.5 (neonatal onset), 32.5 (infant onset), 31 (later onset) and 37 (ASD/ID). Lower severity indices were associated with slightly higher Vineland standardized scores (Supplementary Fig. 1); however, even in the group with a severity index of zero (least severe), the mean Vineland composite score was 50 (SD = 18.7, maximum score = 74). Likewise, in this 'least' severe group, scores for communication (mean = 46, SD = 23), motor (mean = 31, SD = 19), socialization (mean = 54, SD = 16.6) and daily living skills (mean = 49.5, SD = 19) were all extremely low, representing average adaptive behaviour scores >3 SD below the norm-referenced means and consistent with severe to profound impairment.

In comparisons of severe impairments and other features across the four primary phenotype groups (Table 2), the presence of severe communication impairment was similar across the groups, ranging from 72% to 83% ($P = 0.89$). Gross ($P = 0.0002$) and fine motor ($P < 0.0001$) impairments, however, were most common in the neonatal-onset (64% gross motor, 59% fine motor) and infant-onset (67%, 61%) groups but dropped substantially in the later-onset group (21%, 13%) and were present in only one individual in the ASD/ID-only group (8%, 8%). Complete reliance on a feeding tube was reported in 67% of the neonatal-onset group but in only 13% and 20% of the infant- and later-onset groups and in none of the ASD/ID group ($P = 0.0008$). CVI was most common in the neonatal-onset group (63%) and decreased progressively from 44% (infant onset) to 25% (later onset) to 17% (ASD/ID, $P = 0.01$). Likewise, scoliosis was most prevalent in the neonatal-onset group (37%) and decreased to 8% in the ASD/ID group ($P = 0.05$).

The six-point severity index varied substantially across the four main phenotype groups, with the highest (severe) median values found in the neonatal-onset (median = 4, IQR 2–5) and infant-onset (median = 3, IQR 2–4) groups and dropping in the later-onset (median = 1, IQR 1–2) and ASD/ID (median = 1, IQR 1–2) groups ($P = 0.002$).

Primary phenotype and epilepsy-related factors

The occurrence of three seizure types varied substantially by age at seizure onset (neonatal, infant and later; Table 2), atonic (0%, 17% and 43%, $P = 0.0001$), focal motor seizures (42%, 0% and 25%, $P = 0.0009$) and epileptic spasms (22%, 67% and 29%, $P = 0.007$).

Table 2 Primary phenotype by other seizure-related features and non-seizure phenotypes

Parameter	Neonatal onset $n = 27$[a]	Infant onset $n = 18$	Later onset $n = 24$	Autism/ID $n = 12$	P-value[b]
Sex, female	12 (44%)	7 (39%)	10 (42%)	7 (58%)	0.75
Age at study entry, years, median (IQR)	4.7 (1.9–7.9)	4.9 (1.9–10.8)	7.6 (5.1–10.3)	5.0 (4.0–8.3)	0.12
Median Vineland Adaptive Behavior Composite scores, median (IQR)	34 (28–43)	33 (21–45)	31 (26–46)	37 (34–49)	0.57
Non-communicative, if <2 years, severely delayed	16 (73%)	13 (72%)	18 (75%)	10 (83%)	0.89
Wheeled device dependent for mobility or, if <2 years, severely delayed	14 (64%)	12 (67%)	5 (21%)	1 (8%)	0.0002
Does not have purposeful palmer hand grasp	13 (59%)	11 (61%)	3 (13%)	1 (8%)	<0.0001
Requires a feeding tube for all nutrition	10 (67%)	2 (13%)	3 (20%)	0	0.008
Cortical/cerebral visual impairment	17 (63%)	8 (44%)	6 (25%)	2 (17%)	0.01
Scoliosis	10 (37%)	3 (17%)	2 (8%)	1 (8%)	0.05
Severity index, median (IQR)	4 (2–5)	3 (2–4)	1 (1–2)	1 (1, 2)	0.002
Seizures and epilepsy					
Age at seizure onset, median (IQR)	1 day (1–2)	7 months (3–9)	23 months (16–32)	–	–
Seizure type (ever)					
Tonic, clonic, or tonic-clonic	22 (81%)	13 (72%)	21 (88%)	–	0.46
Atonic	0	3 (17%)	10 (43%)	–	0.0001
Myoclonic	5 (19%)	6 (33%)	5 (21%)	–	0.53
Absence	7 (26%)	5 (28%)	9 (38%)	–	0.65
Focal motor	11 (42%)	0	6 (25%)	–	0.0009
Behavioural arrest	4 (15%)	4 (22%)	6 (26%)	–	0.64
Gelastic	4 (15%)	1 (6%)	5 (21%)	–	0.34
Epileptic spasms/West syndrome	6 (22%)	12 (67%)	7 (29%)	–	0.007
Lennox–Gastaut syndrome	6 (22%)	5 (28%)	6 (25%)	–	0.91
Good/excellent seizure response[c]					
Phenytoin ($n = 28$)	17/18 (94%)	0/3 (0%)	0/7 (0%)	–	<0.0001
Lacosamide ($n = 21$)	8/14 (57%)	0/5 (0%)	0/2 (0%)	–	0.01
Carbamazepine ($n = 17$)	5/11 (45%)	1/5 (20%)	0/1 (0%)	–	0.38
Oxcarbazepine ($n = 26$)	6/14 (43%)	2/8 (25%)	1/4(25%)	–	0.63

Note that some variables have missing values for one to three participants.
[a]Four children <1 year of age with neonatal seizure onset and a fifth whose parents did not complete the functional and developmental data form and are excluded from analyses of developmental and functional variables but included for seizure variables.
[b]P-values are based on a χ^2 test for categorical data and Kruskal–Wallis test for ordinal data. Tests are on three degrees of freedom for comparisons that include the autism group and on two for epilepsy-related features.
[c]Good to excellent = significantly reduced seizures or controlled them completely. Denominators for those treated with each drug are provided.

Convulsive (tonic–clonic, clonic or tonic), myoclonic, absence, non-absence behavioural arrests and gelastic seizures did not vary substantially across the three seizure-onset groups (all *P*-values >0.30). Even with a conservative Bonferroni correction, the findings for the three seizure types that did vary would attain conventional significance at $P < 0.05$. A diagnosis of Lennox–Gastaut syndrome occurred in similar proportions across the three epilepsy groups (22%, 28% and 25%, $P = 0.91$), and 8/17 (47%) with Lennox–Gastaut syndrome first had epileptic spasms.

Primary phenotype and response to sodium channel blockers

Of those with epilepsy, 17/18 (94%) in the neonatal-onset group who were treated with phenytoin had a good-to-excellent response, whereas none of those in the infant-onset (0/3) and later-onset (0/7) groups did ($P < 0.0001$, Table 2). For lacosamide, these proportions were 57% in the neonatal-onset group and 0% in both the infant- and later-onset groups ($P = 0.01$). There were non-significant but similar patterns for carbamazepine and oxcarbazepine.

SCN2A variants exhibit functional diversity

Sixty-nine unique *SCN2A* variants were reported in this cohort, including 11 frameshift, 50 missense, six nonsense, one whole-gene deletion and one spice site mutation. Eight variants occurred in two or more participants, including five variants found in two participants each, two variants found in three participants each, and one variant found in four participants. Most variants were *de novo*; however, two were inherited from mosaic (11% and 15.2%) mothers. According to our functional scoring rubric (Table 1), the classification of variant function for 69 unique variants from 81 participants (69v/81p) was as follows: GOF (5v/6p), mixed (12v/13p), WT-like (4v/4p), LOF (17v/25p), severe LOF (12v/12p) and presumed complete LOF (truncating and gene deletion, 18v/20p). The one splice site variant, which could not be modelled, was associated with ASD/ID. The locations of the variants are plotted on a schematic of the $Na_v1.2$ protein shown in Supplementary Fig. 2.

We used automated patch clamp to characterize functionally 48 variants in the adult splice isoform and 24 variants in the neonatal splice isoform. Multiple biophysical properties were assessed for each variant (Supplementary Figs 3–13). Data represent recordings from 6780 individual cells (Supplementary material, Datasets 1 and 2). Figure 1 presents volcano plots summarizing the functional properties of variants with significant differences from WT. For each variant, significant differences in each individual biophysical parameter in comparison to the WT channel were assessed using our scoring rubric to generate a functional classification (Supplementary Table 2). This approach yielded 12 severe LOF variants, 17 LOF variants, five GOF variants, 12 mixed function variants and four variants with properties indistinguishable from WT in our assay (Fig. 2). Of 21 variants

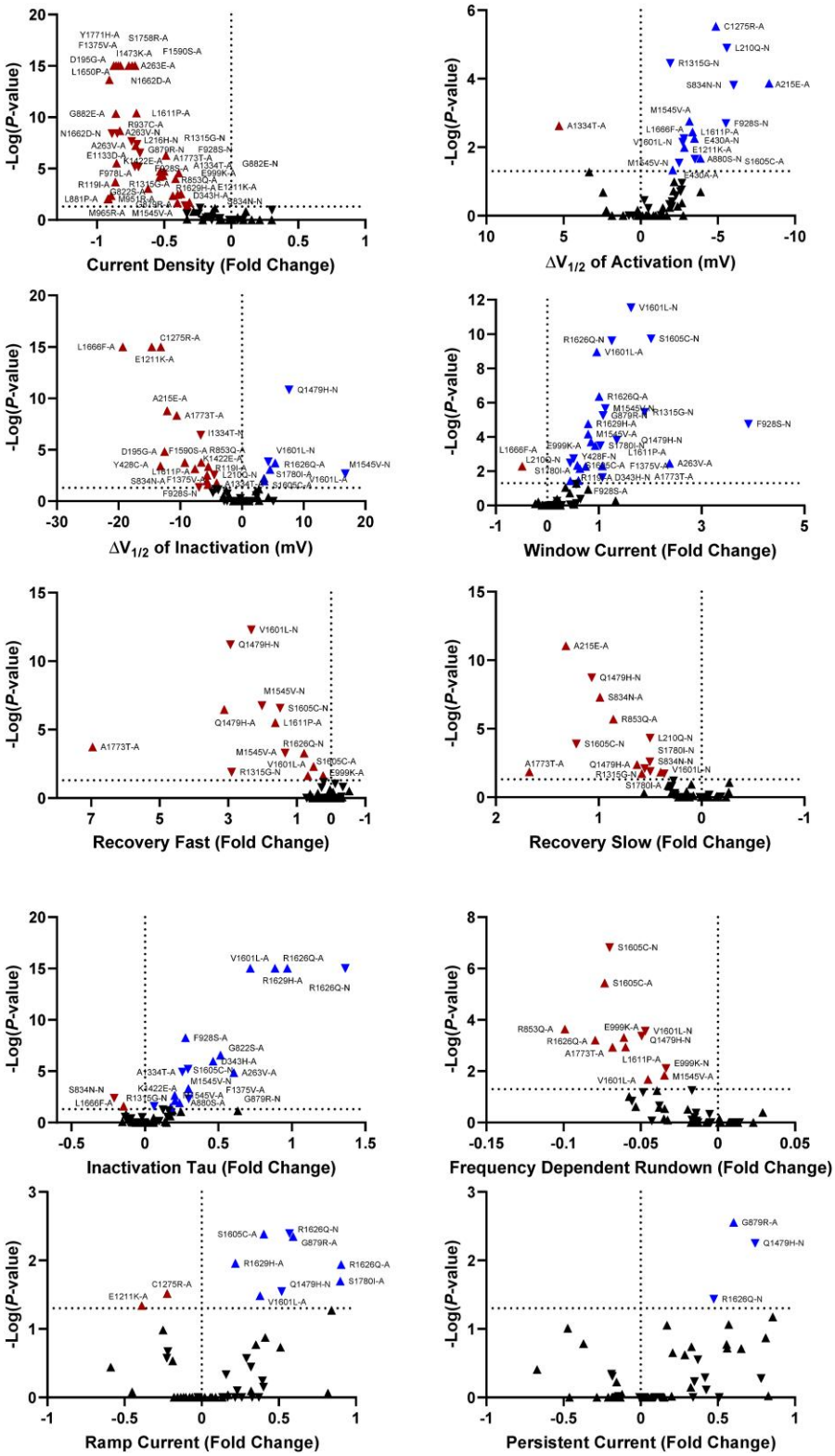

Figure 1 Volcano plots of each of 10 biophysical parameters for *SCN2A* variants of participants in the Clinical Trials Readiness Study. Deviation from wild-type (WT) of disease-associated *SCN2A* variants recorded in the adult (upward triangles) and neonatal (downward triangles) splice isoforms. Only variants significantly different from WT are labelled with variant name, with the suffix -A or -N to indicate splice isoform. Blue symbols denote gain-of-function, and red symbols denote loss-of-function. The horizontal dotted line represents the statistical threshold of $P = 0.05$. Tau = time constant; $V_{½}$ = voltage at half-maximum.

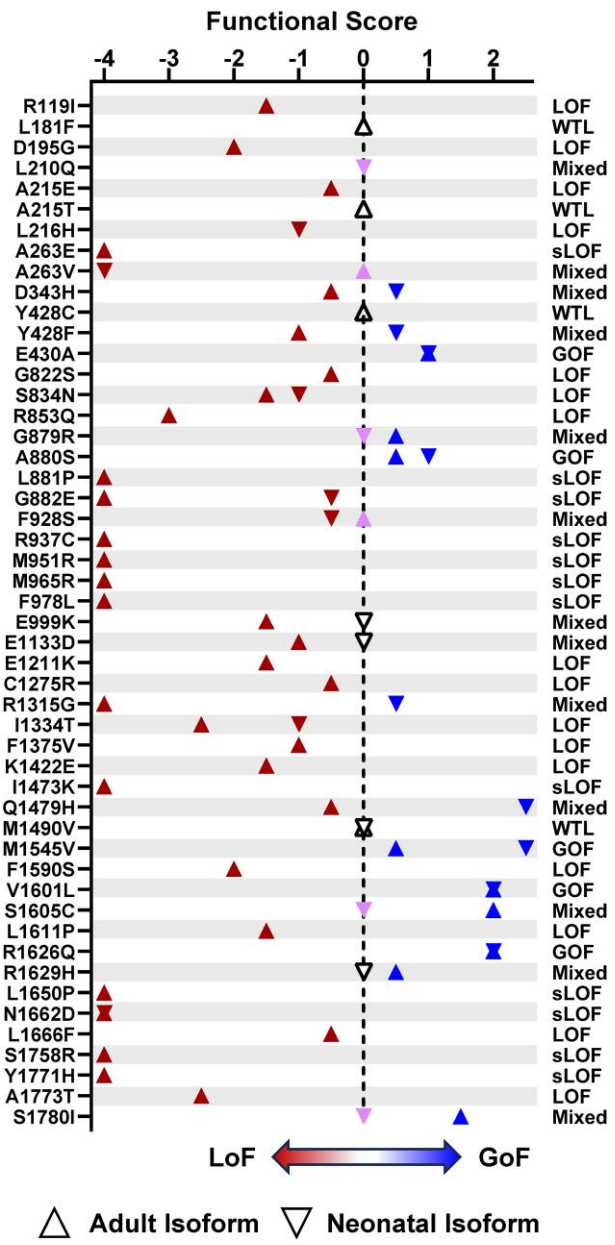

Figure 2 Functional assessment of *SCN2A* Clinical Trials Readiness Study variants. Aggregate functional scores of disease-associated *SCN2A* variants characterized in adult (upward triangles) and neonatal (downward triangles) splice isoforms. Red triangles represent aggregate loss of function. Blue triangles represent aggregate gain-of-function. Purple triangles represent aggregate mixed function from equal, but opposite loss- and gain-of-function scores. Black triangles represent variants that show no functional divergence from wild-type channels. GOF = gain-of-function; LOF = loss-of-function; sLOF = severe loss-of-function; WTL = wild-type like.

expressed in both the neonatal and adult isoforms, 12 had discordant functional classifications affecting 13 participants.

Relationship between primary phenotype and variant function

All children in the neonatal-onset group had missense variants. The proportion with missense variants was smaller in the other groups and represented only one-third of the participants with ASD/ID ($P < 0.0001$; Table 3). All five GOF, all 12 mixed and two of four WT-like variants occurred in the neonatal-onset group (Table 3). In contrast, all participants in the infant-onset ($n = 18$) and ASD/ID ($n = 11$) groups and 22/24 in the late-onset group had LOF variants, severe LOF or truncating variants. The proportion with severe and complete LOF variants increased with age at onset and was greatest in the ASD group ($P < 0.0001$).

Recurrent variants

Complete concordance for primary phenotype was observed in five of eight variants, including the four children with A1773T (Supplementary Table 3). The discordances included D195G (three participants) with two later onset (at 13 and 14 months) and one infant onset (at 11 months); R524X (two participants) with ASD only and later-onset epilepsy, and E1211K (three participants) with infant-onset (9 and 4 months) and later-onset (18 months) seizures. Occurrence of spasms was concordant for five of seven variants, for which all participants had a history of epilepsy, including A1773T (four participants) and D195G (three participants). For the two participants with R524X, one did not have any epilepsy, and the other (later onset) did not have spasms.

Relationship of variant dysfunction to non-seizure phenotypes

Most of the associations between the non-seizure phenotype indicators and impact on channel function were modest or weak and not consistent across indicators (Table 4). The association with female sex ($P = 0.006$) is unexpected, and we suspect that it represents a chance finding. Of the seizure types, epileptic spasms ($P = 0.03$) and atonic seizures ($P = 0.01$) varied with the classification of variant function.

All participants with a reported good-to-excellent seizure control in response to phenytoin had neonatal-onset seizures. In this group, six had GOF, five mixed, two WT-like, two LOF and two severe LOF variants. Of 11 participants with fair-to-poor responses, only one had neonatal onset; of the others, three had infant onset and seven had later onset. Likewise, responses to the other three sodium channel blockers suggested that age at onset and not variant functional classification influenced response to the medication.

Cluster analysis of primary and non-seizure phenotypes and variant function

Initial cluster analyses of primary phenotype, non-seizure severity index and variant function indicated that severe communication impairment should be dropped from the index because it was present in most participants and was not associated with either primary phenotype or variant function. A separate, five-level indicator was subsequently constructed based on the remaining features (mobility, hand use, eating, CVI and scoliosis). All variables were rescaled, and only clusters that contributed ≥5% to the model R^2 were retained. Clustering procedures with variant function, primary phenotype and a five-component severity index yielded a five-cluster solution with an associated R^2 of 0.82 (Fig. 3).

The five clusters were as follows. Cluster 1 ($n = 12$) was composed of individuals with mild non-seizure phenotype indices, mostly later-onset epilepsy, with a few infant-onset cases and moderate LOF *SCN2A* variants. Cluster 2 ($n = 16$) contained individuals with more severe non-seizure indices than in Cluster 1. The group was weighted towards infant-onset epilepsy, with a few later onset. The impact on $Na_v1.2$ channel function was also mostly moderate LOF. Cluster 3 ($n = 22$) was composed of all the ASD/ID patients and the

Table 3 Functional impact on sodium channel by primary phenotype

Phenotype	Neonatal-onset seizures $n = 27$	Infant-onset seizures $n = 18$	Later-onset seizures $n = 24$	Autism/ID without epilepsy $n = 11$[a]	P-value (d.f.)
Gain-of-function ($n = 6$)	6 (100%)	0	0	0	$P < 0.0001$ (15 d.f.) and $P < 0.0001$ (1 d.f.) with Mantel–Haenszel χ^2 for trend
Mixed function ($n = 13$)	13 (100%)	0	0	0	
Wild-type-like ($n = 4$)	2 (50%)	0	2 (50%)	0	
Loss-of-function ($n = 25$)	3 (12%)	11 (44%)	10 (40%)	1 (4%)	
Severe loss-of-function ($n = 12$)	3 (25%)	5 (42%)	1 (8%)	3 (25%)	
Complete loss-of-function (truncation; $n = 21$)	0	2 (10%)	11 (55%)	7 (35%)	

[a]The one splice site variant occurred in the ASD group. That child is not included in this table.

remaining half of those with late-onset epilepsy. The non-seizure severity index was very low (18/22 had a value of zero). All variants were either severe or complete (truncating) LOF. Cluster 4 ($n = 7$) contained only neonatal-onset epilepsy cases with relatively mild non-seizure phenotype scores. All variants were either GOF or mixed function. Cluster 5 ($n = 12$) contained the remaining neonatal-onset cases but with much more severe non-seizure phenotype scores. The variants were largely mixed function, but there were also some LOF and severe LOF variants.

We examined factors not used in clustering with the clusters themselves (Table 5). Severe communication impairment varied substantially among clusters, primarily owing to differences between the two neonatal groups ($P = 0.0005$). In those with epilepsy, some variability in seizure types was observed for atonic ($P = 0.01$) and focal motor ($P = 0.02$) seizures. Epileptic spasms, however, varied considerably across the clusters ($P = 0.0003$) from 0% (Cluster 4) to 75% (Cluster 2, infant onset with moderate LOF variants). Ohtahara syndrome appeared almost exclusively in Cluster 5 ($P = 0.0008$). Recency of last seizure varied substantially across the clusters ($P = 0.0002$); however, most of this effect was attributable to differences between the two neonatal groups. Associations of clusters with phenytoin response reflected the age at seizure onset within the clusters.

Cluster 4 (neonatal, lower non-seizure severity index) had the least severe presentation and course. The average Vineland composite score in this group (available for six) was 52, with a maximum score of 74. The average score of >3 SD below the norm-referenced mean is not consistent with SeLFNIE. Only one variant in that group was inherited from a mosaic parent (11%).

Discussion

Through a strategic collaboration, we extended the understanding of the genotype–phenotype associations for *SCN2A*-RD by systematically characterizing and analysing seizure and non-seizure phenotypes with detailed variant functional classifications. Our findings offer a more inclusive description of the phenotypic landscape and a more thorough evaluation of relationships between variant dysfunction and clinical features that can enable better stratification of affected individuals for inclusion in appropriate clinical trials.

Our approach: (i) refined the primary phenotype to reflect a more granular and developmentally focused approach to age at seizure onset; (ii) developed a separate characterization of non-seizure phenotype to reflect the concept of 'encephalopathy' independently of seizures; and (iii) systematically determined detailed functional properties of a large number of variants found in affected individuals.

Primary phenotype

The modified primary phenotype, which divided age at seizure onset into three developmentally salient groups, resulted in a four-level classification that provided meaningful discrimination between children with respect to the types of seizures and epilepsies and to their degree of non-seizure phenotype impairments and the variant function. This differs from the previous approach using <3 months versus >3 months for seizure onset and, importantly, tt disaggregates neonatal onset from infant onset from later onset and thus aligns with distinctions in common clinical practice for child neurology and epilepsy.[5,8,17] The distribution of seizure onset after 1 year did not allow for further breakdowns into adequately sized groups for analysis, and almost 90% had experienced the first seizure by 2 years of age. In the epilepsies in general, the importance of age at seizure onset is paramount, with distinctions typically made between neonatal, infant and childhood seizure onset.[21,31] From previous series of *SCN2A*-RD patients, these distinctions might not have been feasible; however, with the present sample ($n = 81$), it is clear that there is a neonatal cluster with onset at or very near birth. The only exception, a 34-week preterm infant whose seizure onset was delayed until 19 days, suggested that maturational catch-up to a critical neurodevelopmental period was required for seizure expression. Onset very near birth could also be consistent with prenatal seizure onset; however, reports so far are anecdotal. Prenatal seizures are difficult to diagnosis. Parents were asked about unusual or excessive activity of the fetus prior to birth as part of a medical checklist. Only two mothers endorsed this, and both had children with neonatal-onset seizures. More detailed questioning of mothers would be needed to determine whether abnormal fetal movements representing seizures might be detectable *in utero*.

Non-seizure phenotype

SCN2A-RD has been described as a severe disorder characterized by 'encephalopathy', which variously refers to developmental delays, movement disorders, intellectual disability and several other conditions.[6,7,12,13,18] Systematic characterization of the extent and nature of the developmental and functional impairments, however, has been inconsistent across studies. Data from the Simons Foundation Autism Research Initiative provided some of the more systematic descriptions in a relatively large group of individuals ($n = 64$) with *SCN2A*-RD; however, the quantitative phenotyping in this study focused on developmental assessments and not on functional impairments. Furthermore, some of the terms used in the Simons Foundation Autism Research Initiative study do not reflect standard definitions in

Table 4 Seizure-related features and non-seizure phenotypes by functional impact on sodium channel

Parameter	GOF $n = 6$ (4[a])	Mixed $n = 13$ (11[a])	WTL $n = 4$	LOF $n = 25$ (24[a])	sLOF $n = 12$	Truncation $n = 20$	P-value[b]
Sex, female	0	8 (62%)	0	12 (48%)	8 (67%)	7 (35%)	0.006
Age at study entry, years, median (IQR)	5.6 (2.4–7.9)	4.7 (1.9–7.3)	10.9 (6.9–13.5)	4.6 (1.7–10.8)	7.6 (4.8–13.4)	6.2 (4.3–8.7)	0.25
Noncommunicative	2 (50%)	9 (82%)	2 (50%)	17 (71%)	10 (83%)	16 (80%)	0.61
Wheeled device dependent	1 (25%)	8 (73%)	1 (25%)	13 (54%)	6 (50%)	3 (15%)	0.02
No purposeful palmer hand grasp	2 (50%)	5 (45%)	2 (50%)	12 (50%)	5 (42%)	2 (10%)	0.07
Feeding tube dependent	1 (25%)	5 (45%)	0	6 (25%)	3 (25%)	0	0.01
CVI	4 (67%)	8 (62%)	1 (25%)	12 (48%)	5 (42%)	3 (15%)	0.05
Scoliosis	2 (33%)	4 (31%)	0	5 (20%)	5 (42%)	0	0.01
Severity Index, median (IQR)	1.5 (0–4.5)	4 (2 –5)	1 (0 –3)	3 (2–4)	2.5 (1–5)	1 (1–1.5)	0.02
Seizure types (ever)							
n with epilepsy	6	14	4	23	9	13	–
Seizure onset age, median (IQR)	1d (1–2 d)	1d (1d–1 d)	9.5 mo (2 d–52 mo)	9.5 mo (4–15.5 mo)	3 mo (2 d–9 mo)	23 mo (15–30 mo)	<0.0001
Tonic, clonic, tonic–clonic	5 (83%)	11 (85%)	4 (100%)	16 (67%)	9 (100%)	11 (85%)	0.13
Atonic	0	0	0	6 (25%)	1 (13%)	6 (46%)	0.01
Myoclonic	0	3 (23%)	1 (25%)	6 (25%)	3 (38%)	3 (23%)	0.54
Absence	1 (17%)	2 (15%)	1 (25%)	9 (38%)	5 (56%)	3 (23%)	0.35
Focal motor	1 (17%)	7 (54%)	1 (25%)	4 (17%)	1 (13%)	3 (23%)	0.23
Behavioural arrest	0	2 (15%)	1 (33%)	2 (13%)	3 (38%)	5 (38%)	0.17
Gelastic	1 (17%)	1 (8%)	1 (25%)	3 (13%)	1 (11%)	3 (23%)	0.88
Epileptic spasms	1 (17%)	2(15%)	2 (50%)	14 (58%)	4 (44%)	2 (15%)	0.03
LGS	1 (17%)	3 (23%)	2 (50%)	6 (25%)	3 (33%)	2 (15%)	0.79
Current ASMs (IQR)	1 (0–2)	3 (2–5)	1 (0.5–2)	2.5 (1–4)	3 (2–4)	2 (1–2)	0.17
Good to excellent response[c]							
Phenytoin ($n = 28$)	6/6 (100%)	5/6 (83%)	2/3 (67%)	2/7 (29%)	2/4 (50%)	0/2 (0%)	0.01
Lacosamide ($n = 21$)	2/2 (100%)	3/6 (50%)	1/3 (33%)	1/6 (17%)	1/ 4 (25%)	0	0.21
Carbamazepine ($n = 17$)	1/3 (67%)	3/6 (50%)	0	1/5 (20%)	1/3 (33%)	0	0.77
Oxcarbazepine ($n = 26$)	1/2 (50%)	3/6 (50%)	1/2 (50%)	2/8 (25%)	1/5 (20%)	1/3 (33%)	0.87

Occasionally, there are missing data-points for one or two individuals. CVI = cortical/cerebral visual impairment; GOF = gain-of-function; mixed = mixed function; LGS = Lennox–Gastaut syndrome; LOF = moderate loss-of-function; sLOF = severe loss-of-function; WTL = wild-type-like.

[a]Four children <1 year of age with neonatal seizure onset and a fifth whose parents did not complete the functional and developmental data form and are excluded from analyses of developmental and functional variables but included for seizure variables.

[b]P-values are based on a χ^2 test for categorical data and Kruskal–Wallis test for ordinal data. Tests are on three degrees of freedom for comparisons that include the autism group and on two for epilepsy-related features.

[c]Good to excellent = significantly reduced seizures or controlled them completely. Denominators for those treated with each drug are provided.

practice (e.g. 'cortical blindness' instead of CVI).[13] All the *SCN2A*-RD affected individuals in our study had severe to profound impairments in all domains assessed, as reflected by the near floor-level Vineland-3 composite scores, which indicated function four or more standard deviations below the norm-referenced mean of the instrument. Instead of focusing on scores for non-seizure phenotypes, we used classifiers from the rehabilitation literature, which were modified in collaboration with parents and used in previous work on people with developmental and epileptic encephalopathies.[22-24] This practice is in keeping with the recent US Food and Drug Administration guidance on patient-focused drug development.[32-34] Inclusion of two often-severe non-seizure conditions that were emphasized by parents during design of the survey (scoliosis and CVI) along with a simple classification of any independent versus no independent function in key functional domains provided a simple but robust indicator of non-seizure phenotype severity, which we used to reflect the concept of encephalopathy.[22-24] The strong association of the severity index with primary seizure type was not unexpected and followed what is commonly seen: earlier age at onset is generally associated with worse non-seizure outcomes, especially in the presence of ongoing seizures and depending on aetiology.[35-39]

Variant characterization

The importance of the impact of the *SCN2A* variant on $Na_v1.2$ channel function for disease expression (phenotype) has been suggested in small case series.[12,14,16] Specifically, GOF variants have been associated with 'early'-onset seizures with encephalopathy (<3 months) and sometimes SeLFNIE, whereas LOF variants are more likely to occur in association with ASD/ID and later-onset epilepsy. The evidence concerning variant function has been derived from manual patch-clamp recording assays, and different criteria for assigning GOF or LOF have been adopted in different studies. Our study combined a systematic and expanded phenotyping approach and a standardized high throughput automatic patch-clamp recording platform. This platform provides consistent, statistically robust, systematic determination of multiple functional parameters, any or all of which might be disrupted by a variant.[30] Our findings further highlight the complexities of the effects of genetic variants on overall channel function. To address this complexity, we present a classification scoring rubric (Table 1) that considers all measured biophysical properties in assigning the functional impact of each variant. This approach was designed to uncouple assessments of variant function from subjective impressions informed by a limited number of channel properties. Although this is a systematic

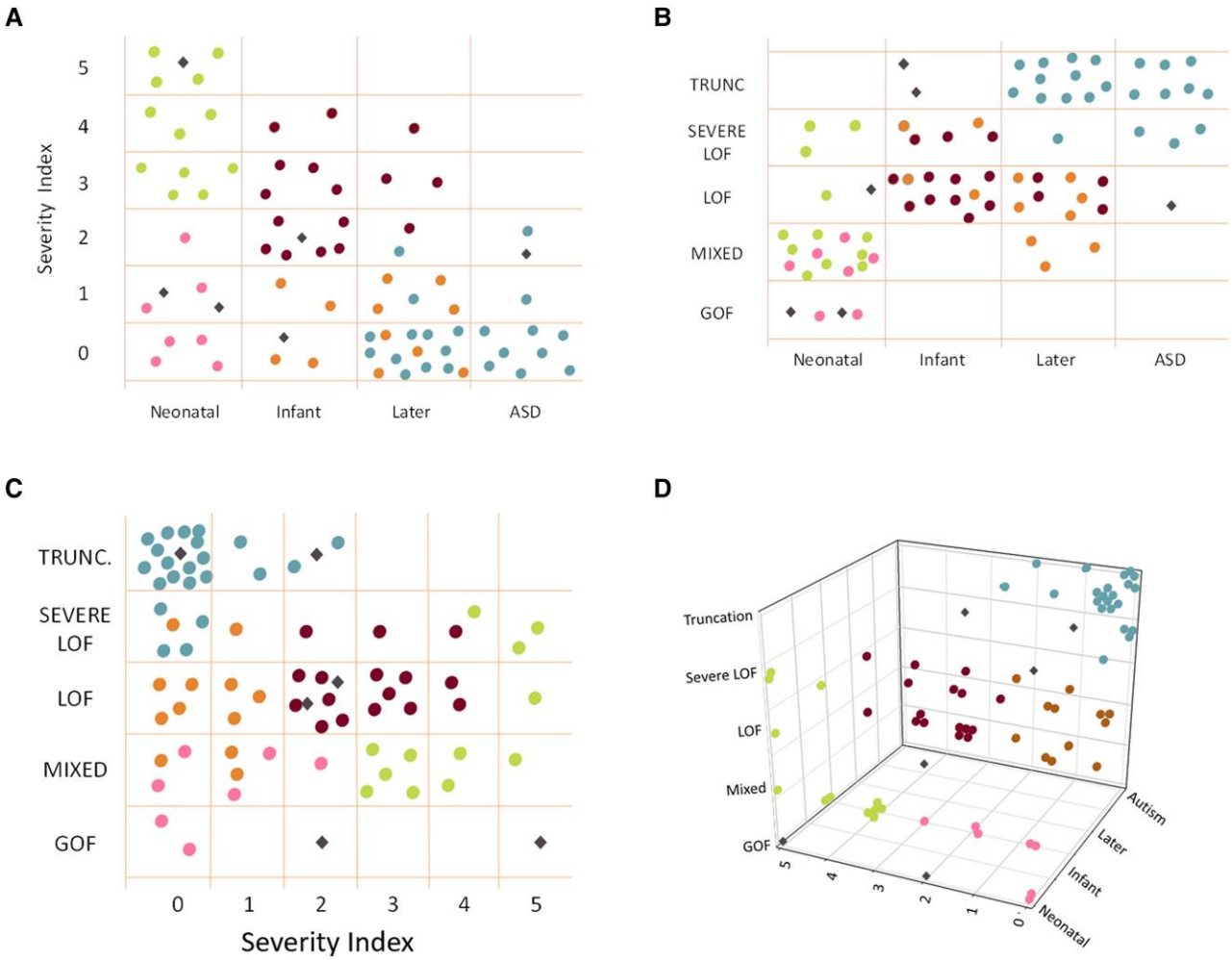

Figure 3 Cluster analysis of clinical phenotypes, severity index, and functional impacts on variant. (**A**) Primary phenotype and severity index. (**B**) Primary phenotype and variant impact. (**C**) Non-seizure severity index and variant impact. (**D**) Three-dimensional representation of the three variables: Cluster 1 (orange circles) contains mostly later-onset epilepsy with mild non-seizure phenotype indices, moderate loss-of-function *SCN2A* variants. Cluster 2 (dark red circles) contains mostly infant-onset epilepsy, more severe non-seizure phenotypes and moderate loss-of-function. Cluster 3 (teal circles) is composed of all of the autism spectrum disorder or intellectual disability (ASD/ID) participants and many late-onset epilepsy cases with severe or complete loss of channel function and very low non-seizure severity indices. Clusters 4 and 5 are composed exclusively of neonatal-onset epilepsy cases. Cluster 4 (pink circles) contains those with milder non-seizure phenotypes and gain-of-function or mixed function variants, whereas as Cluster 5 (green circles) contains individuals with severe non-seizure phenotypes and a range of variant functions, including severe loss of function. Observations that were trimmed by the clustering algorithm are shown as dark grey diamonds.

approach, we do not have data to guide how each property should be weighted. Future studies interrogating neuron models might be valuable in determining the physiological consequences of variants, including which properties are the main drivers of abnormal neural network excitability.

Among the variants we investigated, functional studies were previously reported for 16, and our overall functional assessments of these variants were concordant with previous reports, with a few exceptions. Two prior studies of *SCN2A*-A263V determined that it was GOF,[18,40] whereas our new data show a more mixed functional effect. The designation of GOF in these previous reports was based largely on larger persistent current, although other properties (e.g. smaller peak current density) might offset this effect. We previously adjudicated *SCN2A*-E1422A as a mixed function variant, in contrast to the present study, which assigned this variant as LOF.[41] The difference in this case was attributable to the previous consideration of aberrant ion selectivity. Prior studies of *SCN2A*-E999K indicated GOF,[42] whereas our new data indicate more LOF properties. Discrepancies can be attributed to differences in the cell type used to express variants, presence or absence of overexpressed β subunits, and laboratory-specific techniques.

We did not investigate the functional properties of truncating variants. These variants are predicted to be non-functional; hence, assignment of 'complete' loss of function to truncating variants is a reasonable; however, we acknowledge that variants in the first or last exon might, in theory, escape nonsense-mediated mRNA decay, resulting in expressed truncated $Na_v1.2$ protein with functional capability.[43,44]

Other aspects of variant function (e.g. subcellular localization in neurons) cannot be modelled in the system we used. Therefore, we cannot infer that variants with WT-like functional properties are not disease causing. Expression of variants in neurons, brain organoids and, ultimately, mammalian models, in addition to quantification of gene expression, might enrich our understanding of the impact of variants on channel function, but these are currently slow, low-throughput methods that are not feasible for large

Table 5 Associations of five clusters with other features not included in clustering algorithm

Feature	Cluster 1 *n* = 12	Cluster 2 *n* = 16	Cluster 3 *n* = 22	Cluster 4 *n* = 7	Cluster 5 *n* = 12	*P*-value (4 d.f.)
Severe communication impairment	6 (50%)	14 (88%)	18 (82%)	2 (29%)	12 (100%)	*P* = 0.0007
Seizure types						
Convulsive	9 (75%)	13 (81%)	11 (92%)	6 (86%)	9 (75%)	0.79
Atonic	4 (36%)	3 (19%)	5 (42%)	0	0	0.01
Myoclonic	3 (25%)	5 (31%)	3 (25%)	0	5 (45%)	0.18
Absence	4 (33%)	7 (44%)	3 (25%)	0	4 (34%)	0.16
Focal motor	2 (17%)	1 (6%)	3 (25%)	1 (14%)	7 (64%)	0.02
Behavioural arrest	2 (18%)	3 (19%)	3 (25%)	3 (43%)	1 (9%)	0.55
Gelastic seizures	0	3 (19%)	3 (25%)	2 (29%)	1 (8%)	0.18
Epileptic spasms	5 (42%)	12 (75%)	1 (8%)	0	5 (42%)	0.0003
Syndromes						
Ohtahara	0	1 (6%)	0	0	6 (50%)	0.0008
Lennox–Gastaut	3 (25%)	6 (38%)	2 (17%)	0	5 (42%)	0.13
Other features						
Good to excellent response to phenytoin	0/2	0/6	0/2	5/5 (100%)	6/7 (86%)	<0.0001
Seizures within previous week	5 (42%)	10 (67%)	2 (17%)	0	9 (82%)	0.0002

numbers of variants. Our approach of variant characterization with leading-edge, high-throughput technology applied within a systematically characterized series of patients represents a valuable step in genotype–phenotype studies that have the potential to support future clinical trials.

An important feature of *SCN2A* and other sodium channel genes is developmentally regulated, alternative splicing.[16,45,46] Liang *et al.*[45] demonstrated that a switch between expression of the neonatal and adult isoforms of *SCN2A* occurs after birth in the human brain; however, there is interindividual variability and some expression of the alternative, 'non-preferred' isoform is present at all times. In addition, nothing is currently known about whether and how pathogenic variants might influence the timing or the degree of the switch between these isoforms. For children with onset of seizures in the neonatal period, we determined the impact of variants on channel function in the context of both adult and neonatal isoforms to provide a consensus classification. In previous work based on manual patch-clamp recording, however, it was not always clear which isoform was used for variant expression, and the criteria for determining impact on channel function varied. We note there were eight variants classified as GOF when expressed with one but not the other isoform (four were GOF with the neonatal isoform and four were GOF with the adult isoform). These were classified as 'mixed' for the consensus classification. These nuances will need to be addressed in future models of the functional impact of variants in this and other ion channel genes. Of note, four variants exhibited WT-like channel properties. Other features that we did not assess, such as trafficking and anchoring to the membrane in neurons, might be abnormal for these variants and contribute to pathophysiology. All variants were reviewed for a minimum likely pathogenicity; therefore, we do not believe that these were benign variants.

Emerging phenotype–genotype groups

To identify potential new relationships between phenotype and variant function, we performed a cluster analysis. The first two clusters were similar with respect to the variant function. The first was weighted towards later-onset epilepsy, whereas the second was weighted towards infant onset. The non-seizure severity indices were lower in the first cluster than in the second, which aligns with literature suggesting that earlier-onset seizures are associated with more severe non-seizure outcomes.[35,37,38] The third group contained all of the ASD/ID and half of the later-onset epilepsy patients with almost exclusively mild severity indices and either severe or complete LOF variants. This strongly supports the hypotheses proposed by others that LOF is seen primarily in ASD/ID patients but pulls in later-onset patients who share, with the ASD/ID group, a milder degree of non-seizure severity and a preponderance of truncating and of severe LOF variants. Finally, the neonatal-onset epilepsy patients separated themselves from the others and divided into two clusters mostly defined by non-seizure severity but also, potentially, by the functional impact on their variants. Notably, the cluster with more severe non-seizure phenotypes was more likely to progress to epileptic spasms and more likely to have had seizures in the previous week in comparison to the less severe cluster.

Strengths and limitations

The recruitment for our study through a parent advocacy group is likely to have excluded individuals with SeLFNIE, because their parents would be unlikely to join such a group. Individuals in Cluster 4, although less severe than those in Cluster 5, do not meet criteria for SeLFNIE based on evidence of severe impairment as reflected in their exceptionally low Vineland-3 scores. Furthermore, all but two children had *de novo* variants (inherited from mosaic parents), hence their presentation is inconsistent with a 'familial' syndrome; however, they might represent a downward extension of a wider spectrum of SeLFNIE-related *SCN2A*-RD attributable to *de novo* mutations that has not been fully appreciated. Membership in advocacy groups does skew the sample studied; however, it is unlikely that this would affect the variants themselves, the specific phenotypic features studied or the relationship between the two.

Phenytoin response has been considered a defining part of the *SCN2A* phenotype.[5] Regardless of our classification of channel function, however, response to phenytoin, a sodium channel blocking agent, which should, theoretically, be effective for gain- but not loss-of-function variants, was good to excellent regardless of the variant classification in the neonatal-onset group and was ineffective for anyone with older onset. This suggests that neonatal-onset seizures are associated with GOF properties, whereas LOF properties are more prevalent among epilepsies after the first month of

life, but that those differences were not fully apparent from our functional investigations.

This study, conducted during the coronavirus disease 2019 pandemic, did not involve direct clinical examinations. Although we attempted to obtain medical records from parents, for most participants these were unavailable or inadequate. Consequently, other than original genetic testing reports to verify variants, all information was parent reported. Although this can be viewed as a limitation, it was also a strength, because all information was systematically queried and, insofar as possible, collected with well-developed and validated questions suitable for parent report. Obtaining medical information by parent report alone might pose greater concerns for accuracy and completeness; however, we specifically queried diagnoses, which should provide information reasonably consistent with what would be found in the medical record. One diagnosis, CVI, is often overlooked and not diagnosed. In this case, medical records would not provide any advantage. Existing participants were recruited from among individuals associated with the FamileSCN2A Foundation and do not represent a systematic sample from initial presentation as done in another study, although that study had neither deep phenotyping nor variant analysis.[47] Our study also cannot provide prospectively the trajectory from diagnosis; however, this is common to virtually all currently available studies of *SCN2A*-RD.

Cluster analysis has been used by others to explore meaningful ways to group patients by complex data.[48] Cluster analysis is not used to test hypotheses but to identify patterns in data, which was our intent. Our sample of 81 participants (not all of whom could be included in the clustering process) with systematically characterized variants is probably one of the largest available.

It is possible that in the ASD/ID group some individuals will go on to develop epilepsy; however, the youngest individual in the ASD/ID group was already older than the age at seizure onset for 88% of those with epilepsy. This suggests that any risk of potential misclassification is minimal. Furthermore, the late-onset epilepsy and ASD/ID groups appeared very similar for the most part, and such misclassification would be unlikely to alter our basic findings. Further examination of age at seizure onset might also be warranted; however, our division into three groups aligned well with child development stages, and our numbers did not permit breakdowns into finer categories.

Conclusion

This study represents a unique collaboration between a family advocacy group-sponsored endeavour to understand clinical phenotypes and a large US National Institutes of Health-funded project with the primary aims of determining the functional impacts of ion channel variants. The opportunity to collaborate resulted in a new model for translational research in the rare, monogenic, neurodevelopmental disease field. There is clearly more to understand about the impact of variants and channel function and the implications those variants have for the human phenotype. This study advances that knowledge in many ways and lays out the importance of systematic phenotyping and systematic and detailed characterization of the impact of variants on channel function. The resulting information will be valuable in planning future trials of precision therapies for *SCN2A*-RD and demonstrates the value of advocacy group-led efforts to advance rare-disease research.

Data availability

The clinical data used in this presentation may be accessed for the purpose of further scientific investigation by obtaining a data use agreement with the FamilieSCN2A Foundation, leah.schust@scn2a.org. The functional data are available through the Northwestern University Galter Health Sciences Library PRISM repository (https://doi.org/10.18131/0d35a-fm264; https://doi.org/10.18131/20bch-9qq10). Plasmids used in this study are available from AddGene ($Na_v1.2A$, #162279; $Na_v1.2N$, #209410).

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Competing interests

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Supplementary material

Supplementary material is available at *Brain* online.

References

1. Sugawara T, Tsurubuchi Y, Agarwala KL, et al. A missense mutation of the Na^+ channel α_{II} subunit gene $Na_v1.2$ in a patient with febrile and afebrile seizures causes channel dysfunction. *Proc Natl Acad Sci U S A*. 2001;98:6384-6389.
2. Heron SE, Crossland KM, Andermann E, et al. Sodium-channel defects in benign familial neonatal-infantile seizures. *Lancet*. 2002;360:851-852.
3. Herlenius E, Heron SE, Grinton BE, et al. *SCN2A* mutations and benign familial neonatal-infantile seizures: The phenotypic spectrum. *Epilepsia*. 2007;48:1138-1142.
4. Howell KB, McMahon JM, Carvill GL, et al. *SCN2A* encephalopathy: A major cause of epilepsy of infancy with migrating focal seizures. *Neurology*. 2015;85:958-966.
5. Wolff M, Johannesen KM, Hedrich UBS, et al. Genetic and phenotypic heterogeneity suggest therapeutic implications in *SCN2A*-related disorders. *Brain*. 2017;140:1316-1336.
6. Reynolds C, King MD, Gorman KM. The phenotypic spectrum of *SCN2A*-related epilepsy. *Eur J Paediatr Neurol*. 2020;24:117-122.
7. Richardson R, Baralle D, Bennett C, et al. Further delineation of phenotypic spectrum of *SCN2A*-related disorder. *Am J Med Genet*. 2022;188:867-877.

8. Lauxmann S, Verbeek NE, Liu Y, et al. Relationship of electrophysiological dysfunction and clinical severity in *SCN2A*-related epilepsies. *Hum Mutat*. 2018;39:1942-1956.
9. Sundaram SK, Chugani HT, Tiwari VN, Huq AH. *SCN2A* mutation is associated with infantile spasms and bitemporal glucose hypometabolism. *Pediatric Neurol*. 2013;49:46-49.
10. Leach EL, van Karnebeek CD, Townsend KN, Tarailo-Graovac M, Hukin J, Gibson WT. Episodic ataxia associated with a *de novo SCN2A* mutation. *Eur J Paediatr Neurol*. 2016;20:772-776.
11. Suddaby JS, Silver J, So J. Understanding the schizophrenia phenotype in the first patient with the full SCN2A phenotypic spectrum. *Psychiatr Genet*. 2019;29:91-94.
12. Sanders SJ, Campbell AJ, Cottrell JR, et al. Progress in understanding and treating *SCN2A*-mediated disorders. *Trends Neurosci*. 2018;41:442-456.
13. Berg AT, Palac H, Wilkening G, Zelko F, Schust Meyer L. *SCN2A*-Developmental and epileptic encephalopathies: Challenges to trial-readiness for non-seizure outcomes. *Epilepsia*. 2021;62:258-268.
14. Begemann A, Acuña MA, Zweier M, et al. Further corroboration of distinct functional features in *SCN2A* variants causing intellectual disability or epileptic phenotypes. *Mol Med*. 2019;25:6.
15. Wolff M, Brunklaus A, Zuberi SM. Phenotypic spectrum and genetics of *SCN2A*-related disorders, treatment options, and outcomes in epilepsy and beyond. *Epilepsia*. 2019;60(suppl 3):S59-s67.
16. Ben-Shalom R, Keeshen CM, Berrios KN, An JY, Sanders SJ, Bender KJ. Opposing effects on $Na_V1.2$ function underlie differences between *SCN2A* variants observed in individuals with autism spectrum disorder or infantile seizures. *Biol Psychiatry*. 2017;82:224-232.
17. Berecki G, Howell KB, Deerasooriya YH, et al. Dynamic action potential clamp predicts functional separation in mild familial and severe de novo forms of *SCN2A* epilepsy. *Proc Natl Acad Sci U S A*. 2018;115:E5516-eE5525.
18. Berecki G, Howell KB, Heighway J, et al. Functional correlates of clinical phenotype and severity in recurrent *SCN2A* variants. *Commun Biol*. 2022;5:515.
19. Nesbitt G, Murphy P. CRID—A unique, universal, patient-generated identifier to facilitate collaborative rare disease clinical research. *IMU*. 2022;31:100973.
20. Nesbitt G. CLIRINX Ltd. Clinical Research Informatics. Accessed 31 October 2023. https://clirinx.com
21. Shellhaas RA, Wusthoff CJ, Tsuchida TN, et al. Profile of neonatal epilepsies: Characteristics of a prospective US cohort. *Neurology*. 2017;89:893-899.
22. Berg AT, Gaebler-Spira D, Wilkening G, et al. Nonseizure consequences of Dravet syndrome, *KCNQ2*-DEE, *KCNB1*-DEE, Lennox–Gastaut syndrome, ESES: A functional framework. *Epilepsy Behav*. 2020;111:107287.
23. Berg AT, Kaat AJ, Gaebler-Spira D. Measuring the inch stones for progress: Gross motor function in the developmental and epileptic encephalopathies. *Epilepsy Behav*. 2022;137:108953.
24. Berg AT, Kaat AJ, Zelko F, Wilkening G. Rare diseases–rare outcomes: Assessing communication abilities for the developmental and epileptic encephalopathies. *Epilepsy Behav*. 2022;128:108586.
25. Paulson A, Vargus-Adams J. Overview of four functional classification systems commonly used in cerebral palsy. *Children*. 2017;4:30.
26. Harvey AR, Morris ME, Graham HK, Wolfe R, Baker R. Reliability of the functional mobility scale for children with cerebral palsy. *Phys Occup Ther Pediatr*. 2010;30:139-149.
27. Wood E, Rosenbaum P. The gross motor function classification system for cerebral palsy: A study of reliability and stability over time. *Dev Med Child Neurol*. 2000;42:292-296.
28. Hidecker MJ, Paneth N, Rosenbaum PL, et al. Developing and validating the communication function classification system for individuals with cerebral palsy. *Dev Med Child Neurol*. 2011;53:704-710.
29. Sparrow SS, Cicchetti DV, Saulnier CA. *Vineland-3, vineland adaptive behavior scales*. 3rd edn. Pearson Psychorp; 2016.
30. Thompson CH, Potet F, Abramova TV, et al. Epilepsy-associated *SCN2A* ($Na_V1.2$) variants exhibit diverse and complex functional properties. *J Gen Physiol*. 2023;155:e202313375.
31. Berg AT, Coryell J, Saneto RP, et al. Early-Life epilepsies and the emerging role of genetic testing. *JAMA Pediatr*. 2017;171:863-871.
32. Federal Drug Adminstration. *Patient-Focused Drug Development: Collecting Comprehensive and Representative Input Guidance for Industry, Food and Drug Administration, 2020 Staff, and Other Stakeholders*. US Department of Health and Human Services. Accessed 15 March 2023. https://www.fda.gov/media/139088/download
33. Federal Drug Adminstration. *Patient-Focused Drug Development: Selecting, Developing, or Modifying Fit-for-Purpose Clinical Outcome Assessments Guidance for Industry, Food and Drug Administration Staff, and Other Stakeholders (Draft Guidance)*. US Department of Health and Human Services. Accessed 15 March 2023. https://www.fda.gov/media/159500/download
34. Federal Drug Adminstration. *Patient-Focused Drug Development: Methods to Identify What Is Important to Patients Guidance for Industry, Food and Drug Administration Staff, and Other Stakeholders*. US Department of Health and Human Services 2022. Accessed 15 March 2023. https://www.fda.gov/media/131230/download
35. Vasconcellos E, Wyllie E, Sullivan S, et al. Mental retardation in pediatric candidates for epilepsy surgery: The role of early seizure onset. *Epilepsia*. 2001;42:268-274.
36. Freitag H, Tuxhorn I. Cognitive function in preschool children after epilepsy surgery: Rationale for early intervention. *Epilepsia*. 2005;46:561-567.
37. Cormack F, Cross JH, Isaacs E, et al. The development of intellectual abilities in pediatric temporal lobe epilepsy. *Epilepsia*. 2007;48:201-204.
38. Berg AT, Zelko FA, Levy SR, Testa FM. Age at onset of epilepsy, pharmacoresistance, and cognitive outcomes: A prospective cohort study. *Neurology*. 2012;79:1384-1391.
39. Berg AT, Wusthoff C, Shellhaas RA, et al. Immediate outcomes in early life epilepsy: A contemporary account. *Epilepsy Behav*. 2019;97:44-50.
40. Liao Y, Anttonen AK, Liukkonen E, et al. *SCN2A* mutation associated with neonatal epilepsy, late-onset episodic ataxia, myoclonus, and pain. *Neurology*. 2010;75:1454-1458.
41. Echevarria-Cooper DM, Hawkins NA, Misra SN, et al. Cellular and behavioral effects of altered $Na_V1.2$ sodium channel ion permeability in $Scn2a^{K1422E}$ mice. *Hum Mol Genet*. 2022;31:2964-2988.
42. Miao P, Tang S, Ye J, et al. Electrophysiological features: The next precise step for *SCN2A* developmental epileptic encephalopathy. *Mol Genet Genomic Med*. 2020;8:e1250.
43. Petrić Howe M, Patani R. Nonsense-mediated mRNA decay in neuronal physiology and neurodegeneration. *Trends Neurosci*. 2023;46:879-892.
44. Kurosaki T, Popp MW, Maquat LE. Quality and quantity control of gene expression by nonsense-mediated mRNA decay. *Nat Rev Mol Cell Biol*. 2019;20:406-420.

45. Liang L, Fazel Darbandi S, Pochareddy S, et al. Developmental dynamics of voltage-gated sodium channel isoform expression in the human and mouse brain. *Genome Med*. 2021;13:135.
46. Thompson CH, Ben-Shalom R, Bender KJ, George AL. Alternative splicing potentiates dysfunction of early-onset epileptic encephalopathy SCN2A variants. *J Gen Physiol*. 2020;152:e201912442.
47. Symonds JD, Zuberi SM, Stewart K, et al. Incidence and phenotypes of childhood-onset genetic epilepsies: A prospective population-based national cohort. *Brain*. 2019;142:2303-2318.
48. Cossu A, Lo Barco T, Proietti J, et al. Clinical characteristics of 80 subjects with KCNQ2-related encephalopathy: Results from a family-driven survey. *Epilepsy Behav*. 2023;142:109153.